
Turning Biomass Crops for Biofuels into Commercial Reality

RICHARD B. FLAVELL

Ceres, Inc.

Thousand Oaks, CA

It is now becoming recognized that, in spite of the huge success of converting corn starch into ethanol by its cleavage to sugars followed by fermentation to ethanol, this cannot satisfy the challenges set by the US government to produce 35 billion gallons of biofuels per year by 2017. Instead, the growing of dedicated energy crops and converting their cellulose and hemicellulose to ethanol, or the equivalent, is a more attractive and essential approach. It can produce a much higher ratio of energy output to input than making ethanol from corn. It also avoids the fuel versus food and feed arguments that are being debated at present. Cellulose-derived biofuels are essential to meet the targets set by the US government and aid in the reduction of the rate of increase of CO₂ production in the atmosphere.

The production of biofuels from cellulosic biomass requires a new industry to be born. It requires the production of feedstocks of high-yielding plants, their harvesting, storage and transport to biorefineries. Here they are subjected to thermochemical conversion or treatment to make the cellulose and hemicellulose accessible to degradative enzyme cocktails, incubation with the enzymes or organisms capable of breaking down the polymers to sugars and then fermentation to ethanol or some similar molecule. These industrial chains have to be integrated in specific localities under conditions where each industrial contributor gets enough reward out of the value chain. Because of the complexities in achieving this, many factors have to be put in place ranging from the technical to the political. My contribution to this meeting focuses on what is needed for dedicated energy crops to be developed and available for biofuels production. This is also the focus of Ceres, “The Energy Crop Company” dedicated to energy-crop production.

DEDICATED ENERGY CROPS

There is much discussion about which crop species are likely to be the most efficient at producing large quantities of biomass sustainably with minimal inputs. These crops need to be capable of generating high biomass on a sustainable basis, have a high ratio of

energy output to input, and high proportions of constituent materials that are suited to the downstream processes of conversion to biofuels. Amongst the favorites in the United States are perennials such as switchgrass, miscanthus and sugar cane because their biomass can be harvested year on year from the same root stock; it is not necessary to use fuel to plant them each year. Switchgrass is a native grass that has covered the US prairies. Many different forms are readily available and the US Departments of Energy and Agriculture (DOE and USDA) have had small research programs to evaluate it for biomass production for many years. Miscanthus, on the other hand, is native to Asia, covering temperate to tropical areas. Sugar cane is adapted to the tropics and over-winters only in the extreme south of the United States. All these species, like corn, are C4 grasses; they make higher biomass per unit of light energy absorbed than most flowering plants. They also have a higher water-use efficiency than most plants. The perennial species also have the valuable property of moving their excess nitrogen and other nutrients back down into the roots upon senescence at the end of the growing season, ready for use again in the spring when growth is reinitiated. This means that valuable nutrients like nitrogen fertilizers, which are very energy expensive to make, are conserved in comparison with annual crop-production systems.

Switchgrass and miscanthus have not received much or any attention from breeders and thus are not optimized for large-scale agriculture and the needs for efficient-energy production. Yet the economics of biofuels production from them is critically dependent on the biomass yield and the efficiency of conversion of the cell-wall materials in the biomass to biofuels. This is because the harvesting and transport costs are some 50% of the cost of the feedstock at the biorefinery gate and these costs are heavily influenced by the distances tractors have to go to harvest the fields and the trucks have to go to transport the harvested materials to the biorefinery. Furthermore, current varieties of these species have not been optimized to achieve the required high biomass under the range of environments that will be necessary for such extensive production of biofuels. Therefore plant breeding is essential to realize the government's goals.

PLANT BREEDING TO IMPROVE DEDICATED ENERGY CROPS

What does it take to breed improved varieties of the key biomass crops such that farmers and biorefinery owners can have confidence that the economics of the feedstock production make sense, given the price of oil, *etc.*?

First, there is the need to assemble a comprehensive collection of germplasm that has a large number of variants of the genes in the species and combinations of the variants that program the ability of the species to survive and produce high yields under a range of conditions. These genes and combinations of genes have been assembled by natural selection. In switchgrass there are two broad types—lowland and upland—that are adapted to southerly and more northerly conditions. These, for the most part, have different numbers of chromosomes and so are relatively distinct. Having a selection of the gene variants in both types is essential to be able to make new combinations via breeding that result in the crops being adapted to the range of environments required. For miscanthus it means getting the germplasm from foreign parts such as Asia. Here one must allow

for the conditions laid down by the Convention of Biological Diversity, which ensures that host countries agree to the commercial exploitation of the germplasm and get some return if it is used commercially elsewhere.

Second, one needs an aggressive plant-breeding program. Plant breeding—making new combinations of genes by combining pollen and eggs from different parents and selecting improved plants—is usually a time-consuming process and requires screening of a large number of progeny and sometimes many generations of further crossing and selection. The selection necessarily needs to be done in the diverse environments relevant to where optimized production is required. This is why plant breeding, while brilliantly successful over long time periods, is usually a slow process when judged against the urgent needs of the farmer and processor for rapid improvements. Faster genetic gains can often be obtained by making hybrids between distantly related parents because the genetic deficiencies of one parent can be made up for by the other parent and the combinations of the dissimilar genes often results in improved vigor where the hybrid is better than either parent. Sugar cane and the highest yielding miscanthus are such hybrids. Switchgrass is a natural hybrid.

What traits commonly need to be optimized?

Biomass yield, tons per unit of land, is the number-one trait to be increased for the reasons described above. To gain the most biomass, plants need to grow as long as possible. This means an early start and growing well until frost or harvest time. If seeds need to be got from the crop, then flowering should be late but not too late for good seed set and ripening. If seed is not required, such as in the biomass production fields, then flowering can be as late as possible to allow more biomass to accumulate. Flowering is often conditioned by daylength/latitude and/or temperature. There are well known genes that determine flowering time so there is the opportunity to select plants that have the right combinations of genes to program flowering for different regions.

Other agronomic traits to be optimized are likely to be drought tolerance, nutrient use efficiency, root growth, disease tolerance, as well as all the architectural features that determine the features of the plant that result in high biomass and ease of harvesting.

Another key trait to provide the maximum yield of gallons of biofuel per unit of land is average cell-wall composition and structure. Cellulose and hemicellulose reside in cell-wall complexes that often prevent easy degradation in the biochemical conversion processes. Lignin complexes have evolved in certain cell walls to provide strength and other properties. Lignin provides more energy upon burning, but inhibits biochemical degradation of cellulose and thus it is desirable to optimize both the amounts and structure of lignin in cell walls to optimize the yield of biofuel per ton harvested.

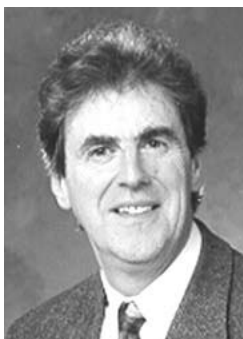
The inhibitory effects of lignin increase the costs of the biochemical process considerably and demand more complex biorefinery construction. Thus assays for energy release and ease of sugar production and fermentation need to be coupled to the plant-breeding and selection processes.

The challenge to produce rapidly high biomass crops with optimum composition to cover the range of environments required is huge. Plant improvement and testing is a relatively slow process. However, today the use of molecular markers of short chromo-

some segments carrying genes of known function offers the opportunity to speed up the selection of improved types. To achieve this requires knowledge of the DNA sequences in the chromosomes of the species, the positions of genes that program desirable traits, and the genetic linkage between variant DNA sequences and preferred versions of genes. Then the DNA markers that signify the preferred genes and gene combinations can be used to monitor the presence of the preferred genes and select the preferred types, without needing to assay the properties of the growing plant so intensively. This can save time and money. Without exploitation of this approach on a large scale, the development of improved plants will be delayed, with substantial consequences for achieving the government's goals. Certain genes conferring valuable properties can be added to a crop as transgenes to speed up plant improvement in ways that are difficult to achieve otherwise. Ceres has amassed a large number of such genes ready for application in dedicated energy crops as breeding programs evolve.

CERES: "THE ENERGY CROP COMPANY"

Ceres is a specialist biotechnology and plant-breeding company committed to providing dedicated energy crops for the biofuels industry, across a range of environments where biorefineries will be built. It is assembling large collections of germplasm for the relevant crops and has established partnerships with other major plant-breeding organizations to develop better crops rapidly. It has in-licensed improved varieties and is bulking up high-quality seed ready for sale to the industry in 2009. This will coincide with the building of the first biorefineries and will provide crops for them to start producing biofuels in 2010. To establish which varieties best suit which environments, Ceres and partners have established many field trials in strategic locations, the results from which will emerge and continue over the coming years. Ceres has established the means of measuring and genetically changing optimum cell-wall composition for dedicated energy crops. It is also linking with biorefineries and other laboratories to evaluate the efficiency of various genetic strains of harvested materials in the various industrial conversion processes. It is deploying molecular markers to build the most efficient breeding processes for these dedicated energy crops and so meet the challenges to supply high-yielding biomass crops where and when they are required.



RICHARD FLAVELL joined Ceres in 1998. From 1987 to 1998, he was the director of the John Innes Centre in Norwich, England, a premier plant and microbial research institute. He has published over 190 scientific articles, lectured widely and contributed significantly to the development of modern biotechnology in agriculture. His research group in the United Kingdom was among the very first worldwide to successfully clone plant DNA, isolate and sequence plant genes, and produce transgenic plants.

Dr. Flavell is an expert in cereal plant genomics, having produced the first molecular maps of plant chromosomes to reveal the constituent sequences. He has been a leader in European plant biotechnology initiating and guiding a pan-European organization to manage large EU plant biotechnology research programs more effectively. In 1999, he was named a Commander of the British Empire for his contributions to plant and microbial sciences.

Flavell received his PhD from the University of East Anglia and is a fellow of EMBO and of the Royal Society of London. He is an adjunct professor in the Department of Molecular, Cellular and Developmental Biology at the University of California-Los Angeles.